JP 05-42917

TITLE OF INVENTION: PROCESS FOR BASE EXCHANGE OF PHOSPHOLIPIDS

WHAT IS CLAIMED IS:

1. A process for the base exchange of a phospholipid with an enzyme, which comprises reacting phosphatidylcholine as the phospholipid with an alcohol selected from the group consisting of serine, ethanolamine, N-methylethanolamine, N,N-dimethylethanolamine, glycerol and monosaccharides in the presence of phospholipase D of Streptomyces origin as the enzyme so as to accomplish the base exchange reaction.

DETAILED EXPLANATION OF INVENTION:

(Related Technical Field)

The present invention relates to a process for the base exchange of phospholipids using an enzyme. In particular, it relates to a process for the base exchange comprising treating phosphatidylcholine with phospholipase D.

(Problem Underlining Invention)

As to the base exchange of phospholipids with enzymes, there is known a process wherein phosphatidylcholine is treated with phospholipase D for the base exchange so as to obtain a phospholipid comprising a desired base [S.F. Yang et al., J.Biol.Chem., 242 (3), 477-484 (1967); R.M.C. Dawson, Biochem. J., 102, 205-210 (1967)].

In said process, the base exchange is carried out mainly by the use of phospholipase D of cabbage origin, but the conversion is less than 13 %. Also, the alcohol usable for the base exchange is limited to a primary alcohol of not more than 5 carbon atoms. Especially, the conversion for nitrogen-containing alcohols is very low and 12 % at the highest. In case of monosaccharides, no base exchange is observed. This invention is directed to improvement in the above respects, i.e. expansion of the range of usable alcohols and attainment of the base exchange of phospholipids with high yields of the desired products.

(Solution for Technical Problem)

The present invention is a process for the base exchange of phospholipids with an enzyme which comprises reacting phosphatidylcholine as the substrate with an alcohol selected from the group consisting of serine, ethanolamine, N-methylethanolamine, N,N-dimethylethanolamine, glycerol and monosaccharides in the presence of phospholipase D of Streptomyces origin.

The phosphatidylcholine usable in this invention may be either a natural product obtained by extraction and purification or a synthetic product.

The phospholipase D of Streptomyces origin usable in this invention is the one obtainable from phospholipase D-producing microorganisms such as Streptomyces chromofoscus or the like and may be commercially available on

the market.

The reaction can be carried out by contacting phosphatidylcholine with an alcohol in the presence of phospholipase D of Streptomyces origin. As the alcohol, there may be used the one chosen from nitrogen-containing alcohols such as serine, ethanolamine, N-methylethanolamine and N,N-dimethylethanolamine as well as polyols such as glycerol and monosaccharides. As the monosaccharides, aldoses and ketoses may be used, and their examples are pentoses such as ribose and arabinose, hexoses such as glucose and fructose, etc.

The solvent to be used for the reaction is water alone or a mixture of water and an organic solvent. Examples of the organic solvent are aliphatic hydrocarbons (e.g. n-heptane, n-hexane, petroleum ether), alicyclic hydrocarbons (e.g. cyclopentane, cyclohexane), ethers (e.g. diethyl ether, tetrahydrofuran), esters (e.g. methyl acetate, ethyl acetate), halogenated hydrocarbons (e.g. carbon tetrachloride, chloroform), etc. When a mixture of water and an organic solvent is used, their proportion may be optional; for instance, the weight ratio of water and the organic solvent may be from 1:1 to 0.1:10. For suppression of the side-reaction and obtainment of the objective product in a high yield, it is preferred to keep the water content in the reaction system not more than 10 % by weight.

The molar ratio of phosphatidylcholine and the alcohol may be appropriately chosen depending on the kind of the alcohol; in general, the alcohol may be used in an amount of 5 - 100 mol to 1 mol of phosphatidylcholine.

The amount of phospholipase D of Streptomyces origin to be used may be selected from the range of 100 - 500 units per 1g of phosphatidylcholine.

After the reagents are charged as above, the resultant mixture is reacted, for instance, at a temperature of 20 to 60℃ while stirring with rotation or supersonic waves for a period of 30 minutes to 5 hours.

(Effect of Invention)

In the present invention, the base exchange reaction of phosphatidylcholine with phospholipase D is carried out by the use of phospholipase D of Streptomyces origin instead of phospholipase D of cabbage origin, whereby a phospholipid substituted with a desired alcohol can be obtained in a high yield. Also, it is possible to accomplish the base exchange with a monosaccharide such as glucose, which could not be accomplished by the use of phospholipase D of cabbage origin. Accordingly, the present invention makes the scope of the alcohol as exchangeable expanded and the conversion of the phospholipid improved.

The present invention will be explained more in details by way of practical embodiments.

Example 1

One hundred ul of a suspension prepared by suspending 40 mg of dipalmitoylphosphatidylcholine in 1 ml of water, 0.5 M acetate buffer (pH 5) (50 ul), ethanolamine (adjusted to pH 5 with 0.5 N HCl) (50 mg), diethyl ether (1 ml),

an aqueous solution of phospholipase D of Streptomyces origin ("Phospholipase DP" manufactured by Toyo Jozo)(50 units/ml)(10 ul) were mixed at 37℃ while stirring at 500 rpm for 1 hour, and after completion of the reaction, the phospholipid was extracted with chloroform.

The extract was subjected to analysis with thin layer chromatography using a developing solvent of chloroform : acetone : methanol : acetic acid : water = 50 : 20 : 10 : 15 : 5 and the Dittmer reagent as a color developing agent, and the composition of the product was determined by densitometry.

As the result, it was revealed that the product comprised 90 % of phosphatidylethanolamine and 10 % of phosphatidic acid.

Example 2

The reaction was carried out in the same manner as in Example 1 but using serine (100 mg) in place of ethanolamine. After completion of the reaction, extraction and analysis were carried out in the same manner as in Example 1. As the result, the product comprised 70 % of phosphatidylserine, 20 % of phosphatidic acid and 10 % of phosphatidylcholine.

Example 3

The reaction was carried out in the same manner as in Example 1 but using glucose (150 mg) in place of ethanolamine. After completion of the reaction, extraction and analysis were carried out in the same manner as in Example 1. As the result, the product comprised 63 % of phosphatidylglucose, 21 % of phosphatidic acid and 16 % of phosphatidylcholine.

Example 4

The reaction was carried out in the same manner as in Example 1 but using glycerol (70 mg) in place of ethanolamine. After completion of the reaction, extraction and analysis were carried out in the same manner as in Example 1. As the result, the product comprised 81 % of phosphatidylglycerol, 11 % of phosphatidic acid and 8 % of phosphatidylcholine.

Example 5

The reaction was carried out in the same manner as in Example 1 but using phospholipase D of Streptomyces chromofuscus (manufactured by Bohringer Mannheim) in place of phospholipase D of Streptomyces origin ("Phospholipase DP" manufactured by Toyo Jozo). After completion of the reaction, extraction and analysis were carried out in the same manner as in Example 1. As the result, the product comprised 83 % of phosphatidylethanolamine, 10 % of phosphatidic acid and 7 % of phosphatidylcholine.

Example 6

The reaction was carried out in the same manner as in Example 1 but using glucose (150 mg) in place of ethanolamine. After completion of the reaction, extraction and analysis were carried out in the same manner as in Example 1. As the result, the product comprised 51 % of phosphatidylglucose, 12 % of phosphatidic acid and 37 % of phosphatidylcholine.

Comparative Examples 1 to 4

The reaction was carried out in the same manner as in Examples 1 to 4 but using phospholipase D of cabbage origin in place of phospholipase D of

Streptomyces origin and adding thereto calcium chloride (10 mg) for activation, the alcohol used being ethanol (Comparative Example 1), serine (Comparative Example 2), glucose (Comparative Example 3) or glycerol (Comparative Example 4).

The analytical results in Examples and Comparative Examples as above are shown in the following table:

	Product	Phosphatidic acid	Phosphatidyl choline
Example 1	90%	10%	0%
Comparative 1	40%	55%	5%
Example 2	70%	20%	10%
Comparative 2	0%	52%	48%
Example 3	63%	21%	16%
Comparative 3	0%	60%	40%
Example 4	81%	11%	8%
Comparative 4	26%	66%	8%
Example 5	83%	10%	7%
Example 6	51%	12%	37%

As understood from the above table, the yields of the products in Examples are markedly higher than those in Comparative Examples for all the alcohols.

In Comparative Examples where phospholipase D of cabbage origin is used, the conversion into phosphatidylethanolamine or phosphatidylglycerol as the exchange reaction product in case of using ethanolamine (Comprative Example 1) or glycerol (Comperative Example 4) as the alcohol is lower than that in Example 1 or 4. It is especially noted that when the alcohol is serine (Comparative Example 2) or glucose (Comparativ3e Example 3), phosphatidylserine or phosphatidylglucose as the product was not obtained.

(19)日本国特許庁(JP) (11)特許出願公告

(12)特許公報(B2) 平5-42917

(51)Int.Cl.⁵	識別記号	庁内整理番号	(24)(44)公告 平成5年(1993)6月30日
C 12 P 13/00		6977-4B	
//(C 12 P 13/00			
C 12 R 1:465)			

発明の数 1 (全3頁)

(54)発明の名称 リン脂質の塩基交換反応法

(21)特願 昭61-180023 (22)出願 昭61(1986)8月1日

(23)公開 昭63-36790 (43)昭63(1988)2月17日

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(56)参考文献 特開 昭58-152431(JP,A) 特公 昭52-39918(JP,B2)

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(57)特許請求の範囲

1 酵素を利用してリン脂質の塩基交換反応を行うに際し、基質としてホスファチジルコリンを用い、セリン、エタノールアミン、N-メチルエタノールアミン、N,N-ジメチルエタノールアミン、グリセロールおよび単糖の群から選ばれるアルコールを、ストレプトマイセス属由来のホスホリパーゼDの存在下に反応せしめ、塩基交換を進行させることを特徴とするリン脂質の塩基交換反応法。

発明の詳細な説明

(産業上の利用分野)

本発明は酵素を利用したリン脂質の塩基交換反応法に関し、特にホスファチジルコリンにホスホリパーゼDを作用させる塩基交換反応法に関する。

(従来の技術と発明が解決しようとする問題点)

酵素を利用したリン脂質の塩基交換反応において、ホスファチジルコリンにホスホリパーゼDを作用させ、塩基交換反応法により目的とする塩基を持つリン脂質を製造する技術は公知である。〔S.F.Yang, et al., J.Biol.Chem.242, (3) 477-484(1967)〕、〔R.M.C.Dawson, Biochem. J. 102, 205~210(1967)〕

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これらの技術では、主としてキャベツ由来ホスホリパーゼDを用いて塩基交換反応を行っているが、その変換率は13%以下であった。また交換反応に使用できるアルコールは、炭素数5以下の一級アルコールに限られていた。特に、含窒素アルコールに関しては変換率が非常に低く、高いものでも12%であった。また、単糖については交換反応が認められなかった。本発明は、これらの点を改善し、使用できるアルコールの範囲を広げ、しかも高収率で目的物が得られるリン脂質の塩基交換反応法を提供することを目的とする。

(問題点を解決するための手段)

本発明は、酵素を利用してリン脂質の塩基交換反応を行うに際し、基質としてホスファチジルコリンを用い、セリン、エタノールアミン、N-メチルエタノールアミン、N,N-ジメチルエタノールアミン、グリセロールおよび単糖の群から選ばれるアルコールを、ストレプトマイセス属由来のホスホリパーゼDの存在下に反応せしめ、塩基交換を進行させることを特徴とするリン脂質の塩基交換反応法である。

本発明で使用するホスファチジルコリンは、天

ミリモル加え、その他の反応条件、操作は実施例1と全く同様に行った。

分析結果は、ホスファチジルグルコース53%、ホスファチジン酸21%、ホスファチジルコリン16%であった。

実施例 4

エタノールアミンのかわりにグリセロールを70ミリモル加え、その他の反応条件、操作は実施例1と全く同様に行った。

分析結果は、ホスファチジルグリセロール81%、ホスファチジン酸11%、ホスファチジルコリン8%であった。

実施例 5

ストレプトマイセス属由来のホスホリパーゼD(東洋紡績㈱製のホスホリパーゼDP)のかわりにストレプトマイセス・クロモホスカス由来のホスホリパーゼD(ベーリンガーマンハイム社製)に変えた以外は、実施例1と同様の組成、条件で反応を行った。反応終了後実施例1と同じ操作にて抽出、分析を行った。

その結果、ホスファチジルエタノールアミン83%、ホスファチジン酸10%、ホスファチジルコリン7%であった。

実施例 6

実施例5において、エタノールアミンのかわりにグルコースを150ミリモル加えた以外は、同様の組成、条件で反応を行った。反応終了後実施例1と同じ操作にて抽出、分析を行った。

その結果、ホスファチジルグルコース51%、ホスファチジン酸12%、ホスファチジルコリン37%であった。

比較例 1～4

ストレプトマイセス属由来のホスホリパーゼDのかわりにキャベツ由来ホスホリパーゼDを用い、活性化のみに塩化カルシウムを10ミリモルを加えた以外は、添加アルコールとしてエタノールアミン（比較例1）、セリン（比較例2）、グルコース（比較例3）、グリセロール（比較例4）を用い、実施例1～4までと同様に行った。

実施例および比較例の分析結果を下表に示す。

表

	生成物	ホスファチジン酸	ホスファチジルコリン
実施例1	90%	10%	0%
比較例1	40%	55%	5%
実施例2	70%	20%	10%
比較例2	0%	52%	48%
実施例3	63%	21%	16%
比較例3	0%	60%	40%
実施例4	81%	11%	8%
比較例4	26%	66%	8%
実施例5	83%	10%	7%
実施例6	51%	12%	37%

表から明らかなように、実施例では、比較例よりも、すべて各アルコールについて著しく高収率で目的物が得られる。

これに対して比較例では、キャベツ由来ホスホリパーゼDを使用したので、添加アルコールがエタノールアミン（比較例1）およびグリセロール（比較例4）の場合、交換反応による生成物、ホスファチジルエタノールアミンおよびホスファチジルグリセロールへの変換率が実施例1および4に比較して低い。特に、セリン（比較例2）およびグルコース（比較例3）の場合には、生成物のホスファチジルセリンおよびホスファチジルグルコースが全く得られなかった。